compared with that of cellulose is substantiated by the evolution data for oxidized starch. Figure 1 discloses that the rate of evolution appeared logarithmic for the first three hours, followed by a slow linear evolution for two and one-half hours. Bearing in mind that the other polyuronic acids examined are made up essentially of long, unbranched chains, whereas starch is composed, in part at least, of branched chains assumed to involve the C_6 hydroxyl group, we find that interpretation of the velocity constant becomes difficult and speculative. Thus, the data may offer further evidence that starch is structurally more heterogeneous than cellulose, celluronic acids, purified pectic acid, or alginic acid.

There appears to be nothing in the carbon dioxide evolution data to contradict the previously published suggestion that celluronic acids are composed of anhydro-D-glucose and D-glucuronic acid units in varying amounts, dependent on the degree of oxidation.

Acknowledgment.—We wish to acknowledge the assistance of Dr. Delbert D. Reynolds in preparing the D-galacturonic acid monohydrate and D-gluconic acid. The method described herein for preparing the latter was developed by him.

Summary

1. Kinetic data on the evolution of carbon dioxide from celluronic acids is submitted as evidence that cellulose, highly oxidized with nitrogen dioxide, is composed essentially of β-D-glucuronic acid units and unchanged D-glucose units.

2. Reaction velocity constants are given for evolution of carbon dioxide from D-glucuronic acid, D-galacturonic acid, L-ascorbic acid, pectic acid, alginic acid, celluronic acids and oxidized starch.

3. The close relationship existing between the rates of acid decomposition of monomeric and corresponding anhydro-polymeric acids is discussed.

4. With the exception of L-ascorbic acid, the nonuronic organic acids tested as reference compounds evolved small amounts of carbon dioxide linearly.

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[COMMUNICATION NO. 1098 FROM THE KODAK RESEARCH LABORATORIES]

Investigation of the Properties of Cellulose Oxidized by Nitrogen Dioxide. III. The Reaction of the Carboxyl Groups of Polyuronides with Calcium Acetate[1]

BY P. A. MCGEE, W. F. FOWLER, JR., AND W. O. KENYON

Numerous investigators of oxidized celluloses have used the calcium acetate method to characterize their products. It was first used to measure the residual acidity of cellulose itself[1a] and has since been employed in determining the equilibrium between cellulose and salts.[2] Calcium acetate is now utilized to estimate the carboxyl groups in celluloses which have been oxidized with nitrogen peroxide (NO_2—N_2O_4),[3,4] *i.e.*, celluronic acids.[5] The present study is to define and delimit the interpretation to be given to carboxyl values so obtained. The object is to use the calcium acetate method to investigate further the structure of celluronic acids rather than to develop it as an analytical procedure. Alginic acid, a naturally occurring polyuronide consisting of β-anhydro-D-mannuronic acid units, is used as a reference material against which celluronic acid, nitrogen tetroxide-oxidized starch, and other natural and synthetic polyuronides may be compared.

Experimental

The alginic acid was liberated by the addition of hydrochloric acid to commercial sodium alginate solution, the free acid being precipitated as swollen gelatinous particles. These were leached in successive changes of distilled water, ethanol, and ether, and finally air-dried. The product, in a flaky condition, was ground in a Wiley cutting mill to 60-mesh size. Moisture was determined just prior to use by the Karl Fischer technique. All results are calculated to the dry basis.

Anal. Calcd. for alginic acid $(C_6H_8O_6)_n$: C, 40.9;

TABLE I

EFFECT OF REACTION TIME ON CARBOXYL CONTENT OF ALGINIC ACID BY CALCIUM ACETATE

Reaction time, hr.	% COOH on dry basis	Mean % COOH (dry)
0.5	23.10	23.05
0.5	23.00	
1.25	23.10	23.12
1.25	23.14	
2.0	23.00	23.05
2.0	23.10	
4.0	23.10	23.04
4.0	22.98	
7.0	22.87	22.90
7.0	22.94	
16.0	23.25	23.13
16.0	23.01	
		Average 23.05

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[1] Presented before the Division of Cellulose Chemistry at the 110th Meeting of the American Chemical Society, September, 1946, Chicago, Illinois.

[1a] M. Ludtke, *Biochem. Z.*, **233**, 25 (1931); **268**, 372 (1934); **285**, 78 (1936); *Z. angew. Chem.*, **41**, 650 (1935).

[2] Heymann and Rabinov, *J. Phys. Chem.*, **45**, 1152 (1941).

[3] Yackel and Kenyon, THIS JOURNAL, **64**, 121 (1942).

[4] Unruh and Kenyon, *ibid.*, **64**, 127 (1942).

[5] Unruh and Kenyon, *Textile Research*, **16**, 1 (1946).

H, 4.54; COOH, 25.56. Found: C, 39.65; H, 4.43; uronic COOH 25.30% (av. 2 determinations).

The calcium acetate analytical method was the same as that employed by Yackel and Kenyon,[5] except that the reaction mixtures were stored in a constant-temperature bath at 25.2 to 25.3°. Results in a time series are summarized in Table I.

Experiments were conducted in which swelling of the alginic acid was carried as far as possible. Duplicate samples of 0.5000 g. were placed in 50 cc. of carbon dioxide-free water and heated at 70° for twenty-four hours. The alginic acid particles were enormously swollen. Then the samples were cooled to room temperature, the usual amounts (30 cc. of 0.5 *N*) of calcium acetate solution were added, and the mixture was permitted to stand for one hour at 25.2 to 25.3°, and titrated. Results are given in Table II.

Table II

Effect of Prolonged Swelling of Alginic Acid in Hot Water

Reaction time. hr.	% COOH on dry basis	Mean % COOH
1.0	23.16	23.155
1.0	23.15	

Titration curves were made with samples which had reacted with calcium acetate for one hour. The results on duplicate 0.5-g. samples are tabulated in Table III, including data calculated from the observed phenolphthalein end-point.

Table III

Electrometric Titration Data for Alginic Acid

Reaction time. hr.	*p*H of equilibrium	% COOH on dry basis	Mean % COOH
1.0	8.79	23.12	23.12
1.0	8.60	23.12	
Electrometric titrations; end-points by graphic solution			
1.0	8.20	22.98	23.05
1.0	8.45	23.12	
Electrometric titrations to phenolphthalein end-points			

Analysis of Celluronic Acid.—The celluronic acid employed was a sample of surgical gauze oxidized with nitrogen peroxide (NO_2–N_2O_4) vapors[6] at the Tennessee Eastman Corporation. The sample had the following analysis, the calculated carbon and hydrogen values being derived from the uronic acid value, assuming the remainder of the chain molecule to be composed of anhydro-glucose units: calcd.: C, 41.50; H, 4.87. Found: C, 42.55; H, 4.81; uronic COOH, 21.02; N, 0.09.

Samples of this celluronic acid gauze were ground to pass a 60-mesh screen and analyzed by the calcium acetate method in a manner similar to that for alginic acid; the time intervals were indicated in Table IV.

Table IV

Effect of Reaction Time on Carboxyl Content of Celluronic Acid by Calcium Acetate Method

Reaction time. min.	% COOH on dry basis		Mean % COOH on dry basis
0	19.93	19.76	19.85
5	19.81	19.85	19.83
10	19.65	...	19.65
15	19.81	19.85	19.84
20	19.79	19.79	19.79
25	19.79	19.76	19.77
			Average 19.79

A series of celluronic acids, prepared by oxidizing cellulose to varying degrees in a carbon tetrachloride-nitrogen peroxide[7] mixture, were analyzed for carboxyl by calcium acetate, in the same manner as the celluronic acid gauze, titrating immediately after mixing the substances together. In addition, oxidized starch, pectic acid, D-galacturonic acid, and L-ascorbic acid were analyzed by the same procedure. Data are summarized in Table V.

Table V

Calcium Acetate Carboxyl Values for Various Substances

Substance	% COOH (dry basis) Ca(OAc)$_2$ method	% COOH (dry basis) CO$_2$ evolution	% N dry basis
Celluronic acid (A)	4.19	7.34	0.63
Celluronic acid (B)	6.66	10.01	.03
Celluronic acid (C)	7.72	9.86	.00
Celluronic acid (D)	8.50	11.89	.59
Celluronic acid (E)	10.50	10.86	.68
Celluronic acid (F)	16.14	22.31	.96
Celluronic acid (G)	21.68	23.80	.20
Oxidized starch	20.52	23.38	.36
Pectic acid	21.25	21.47	
D-Galacturonic acid[a]	22.79	23.35	
L-Ascorbic acid	27.44	30.41	

[a] Theoretical COOH, 23.19%; for a polyanhydrouronic acid, 25.56%.

Discussion

It is apparent from the data of Table I that highly purified alginic acid, when analyzed by the calcium acetate method, did not give the theoretical carboxyl value of 25.56%. The carboxyl values, for the same substance, by the carbon dioxide evolution method, are seen to be approximately equal to the calculated carboxyl value for polyanhydro-β-D-mannuronic acid. Table I also shows that the reaction between alginic acid and the calcium acetate is rapid, especially considering the fact that the reaction mixture is heterogeneous. The values from half to sixteen hours reaction time agree within experimental error. To ascertain whether these values represent the maximum obtainable under far more vigorous conditions, the alginic acid was preswollen to present the largest possible surface to reactants. This pretreatment of the alginic acid did not alter the carboxyl value appreciably (Table II).

The carboxyl values obtained by titrating the liberated acetic acid to the phenolphthalein end-point (Table III) agree well with the more exact value obtained by graphic determination of equivalence. The use of potentiometric titration in the calcium acetate method is preferred for research because the end-point obtained from a titration curve is determined by several experimental observations, *i.e.*, the points on the curve in the vicinity of equivalence, whereas an indicator end-point is based on a single observation.

The wide use of the calcium acetate analysis in characterizing celluronic acids made it advisable

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(6) Yackel and Kenyon, U. S. Patent 2,232,990, Feb. 25, 1941.

(7) Yackel and Kenyon, U. S. Patent Application no. 571,012.

to ascertain the precision obtainable at very short reaction times and for immediate titration. The results summarized in Table IV indicate that the reaction of calcium acetate with celluronic acid is very rapid, as all the values agree within experimental error. It seems established, then, that at least for finely ground samples, the reaction liberating acetic acid is so rapid that an aliquot of the filtered reaction mixture may be titrated as soon as desired after the reactants have been combined.

The sample of celluronic acid used in the time series discussed above showed a higher carboxyl value by the carbon dioxide evolution method than by calcium acetate. This phenomenon appears to apply generally to polyuronides, including seven different celluronic acids, oxidized starch and pectic acid (Table V). The same observation applied to alginic acid. Apparently, calcium acetate and uronic acid determinations do not necessarily measure the same chemical entities.

The differences between the carboxyl values obtained by the calcium acetate and those from the carbon dioxide method, as shown in Table V, are noteworthy. We have shown earlier[8] that cellulose nitrate decomposes under the conditions of the latter method to produce carbon dioxide. The presence of combined nitrate groups in certain of the celluronic acids may have produced a part of the divergence in carboxyl values shown in Table V, but we do not believe this accounts for all of the difference. Celluronic acids B and C possessed extremely small amounts of combined nitrogen yet showed real analytical differences. Similar differences were obtained with alginic acid where combined nitrate groups are surely not a factor. We believe that the rigorous hydrolytic conditions of the uronic acid method decomposes all carboxyl groups in uronic acid units, whether free or bound, while the calcium acetate method operating at a *p*H of 5 to 6 measures only the free carboxyl groups. In the absence of combined nitrate groups, this difference represents bound or potential carboxyl which may be in the form of intermolecular anhydride or ester groups or as intramolecular lactone. Many celluronic acids have been observed whose calcium acetate carboxyl values were below that considered necessary for solubility in dilute alkali. Such acids did dissolve, however, and their higher uronic acid carboxyl values were compatible with such behavior. Potential carboxyls of the type just described would be susceptible to alkaline hydrolysis and therefore should become available for solubility effects. Additional evidence for such structures is presented in the succeeding paper of this series.

Summary

1. The calcium acetate method of carboxyl analysis did not produce theoretical values for celluronic acid or other synthetic or natural polyuronides examined.

2. Finely divided samples of celluronic acids may be titrated with precision, using calcium acetate immediately after the reactants are combined.

3. Inter- or intramolecular dehydration could account for the difference between carboxyl content as found by calcium acetate determination and the carbon dioxide evolution method.

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[8] Taylor, Fowler, McGee and Kenyon, THIS JOURNAL, **69**, 342 (1947).

[COMMUNICATION NO. 1099 FROM THE KODAK RESEARCH LABORATORIES]

Investigation of the Properties of Cellulose Oxidized by Nitrogen Dioxide. IV. Potentiometric Titration of Polyuronides[1]

BY C. C. UNRUH, P. A. MCGEE, W. F. FOWLER, JR., AND W. O. KENYON

Celluronic acids, prepared by the oxidation of cellulose with nitrogen dioxide,[1a] have been analyzed for carboxyl groups by several methods but only a few have structural significance. Titration with dilute alkali or pyridine–water solutions (or suspensions) produced erratic results. The reaction was physically heterogeneous for slightly oxidized samples and homogeneous for highly oxidized materials.[2] The modification of titrating a strongly alkaline aqueous pyridine solution of the celluronic acid gave results in excess of theory.[2]

The use of calcium acetate, introduced by M. Ludtke,[3] for determination of carboxyl groups in cellulose gave highly reproducible results.[1a] This method depends on the exchange of ions between celluronic acid and calcium acetate liberating an equivalent amount of acetic acid. It is, in such cases, a heterogeneous reaction. The method appears to measure free carboxyl groups, though it may not show potential uronic acid carboxyl groups.[4]

Application of the carbon dioxide evolution method[1] to celluronic acid invariably produced higher carboxyl values than did the calcium acetate method. The rate of evolution has been

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[1] Presented before the Cellulose Chemistry Division at the 110th Meeting of the American Chemical Society, September, 1946, Chicago, Illinois.

[1a] Yackel and Kenyon, THIS JOURNAL, **64**, 121 (1942).

[2] Unruh and Kenyon, *ibid.*, **64**, 127 (1942).

[3] M. Ludtke, *Z. angew. Chem.*, **41**, 650 (1935).

[4] McGee, Fowler and Kenyon, THIS JOURNAL, **69**, 347 (1947).